International Journal of Bio-Technology and Research (IJBTR)
ISSN 2249-6858
Vol.2, Issue 3 Dec 2012 21-28

SSR MARKER ANALYSIS OF F_3 MAPPING POPULATION INVOLVING POKKALI AND IR28 FOR SALT TOLERANCE

R. RAJANI[1] & R. ELANCHEZHIAN[2]

[1]K.S.R College of Arts and Science, Tiruchengode - 637 209, Tamilnadu, India

[2]Central Agricultural Research, Institute (ICAR), PB No. 181, Port Blair-744101, A & N Islands

ABSTRACT

Salinity is one of the major abiotic stresses detrimental to crop production. Amazingly about 25 million acres of land are lost due to salinity each year throughout the world. Nearly 47% of saline, 20% of sodic (alkali) and 7% of acid sulphate soils of the tropical Asia comes from India. In India, there are about 12 mha of land affected with salinity and alkalinity and nearly 4 mha affected with salinity alone. Under such scenario, increased salt tolerance of crops is needed to sustain food production in many regions in the world including India. In the present study, molecular profiling of F_3 mapping population generated from a cross involving salt tolerant Pokkali and salt susceptible IR28 were done using micro satellite markers (SSR) and phenotype were studied under simulated conditions. Assessment of their genetic diversity through molecular profiling involving SSR markers and linking salt tolerance profile showed salt tolerance is a polygenic character. The status of phenotypic marker was also found through the present study. Salt tolerant gene(s) in F_3 generation was tagged along with concurrent phenotype in response to stimulated saline condition. The result showed ample polymorphism. Considering the degree of polymorphism among the mapping population, it was concluded that SSR markers are efficient in tagging gene(s) for salt tolerance in rice. Further, such technology will be useful in marker aided selection in crops towards improving tolerance to salt or any kind of abiotic stress prevailing in different agro-ecological regions of India.

KEY WORDS: SSR markers, Molecular Tagging, genes, Salt Tolerance, Rice

INTRODUCTION

Rice is the principal cereal crop of Asia. It faces innumerable biotic and abiotic stresses under the growing environment. Day by day such threats are inflating due to resistance breakdown of popular high yielding varieties and development of newer saline and alkaline zones due to over exploitation of ground water for irrigation. Salinity is one of the serious abiotic stresses making substantial yield loss annually. The problem has been included in the priority list of many countries such as “National Agricultural Research Programme” of Japan, China, India and Malaysia etc. as well as in the Rockefeller foundation’s “Rice improvement programme”. Abiotic stress generated by mineral salts affected a considerable portion of the arable land; they ranked second after moisture stress (Table 1). In India, about 12 mha of land are affected with salinity and alkalinity (Yadav and Gupta, 1984) and nearly 4 mha affected with salinity alone (Paul and Ghosh, 1986). High concentration of NaCl and other salts are the main limiting factors in rice cultivation on very large area across diverse ecosystem. To extend the rice cultivation in salt affected and marginal areas, breeding of salt tolerant varieties are felt essential. Coastal lands have complex soil with hydrological problems such as acid, acid sulfate, and/or peat soils with nutrient deficiency and mineral toxicities. Soils with neutral soluble salts adversely affect growth and performance of crops. The salts involved are mainly chlorides and sulphates of sodium, calcium, magnesium and potassium. They are indigenous to the soil in coastal areas. The soil solution of wet saline soil has water potential of -24

bars, or higher. Plants in higher salinity fail to meet transcriptional demands, and may ultimately wilt and desiccated. NaCl adversely affects the activity of enzymes of the glycolytic pathway. Salinity affects oxidative phosphorylation and ATP/ADP ratio in the root tissue and reduces the supply of energy. Cell wall may be involved in salt injury, partly by interaction of Na^+ with the cell wall bound ions such as Ca^{2+}. Because of these multiple stresses, hybrid varieties must be evaluated with the naturally occurring soil and climatic conditions for improving selection efficiency. However this process is time consuming and delays breeding progress.

Table 1: Percent of Arable Land Affected by Various Abiotic Stresses

Abiotic Stress	Fraction (%) of Arable land
Drought	26
Mineral (Toxicity/deficiency)	20
Freezing	15
No stress	10

A substantial amount of land across the globe remains idle due to salinity. The problem is increasing in a compounding rate owing to a variety of natural and manmade causes. An area of 4000 ha of soil affected with salinity existed in Andaman and Nicobar Islands having potential to produce an additional 12000 tons of rice (Mandal et al 2004). In this coastal area development of a promising salinity tolerant line through hybridization and selection would take 8-10 years. This is aggravated by the fact that in areas where salinity or other stresses are found, only one crop per season is possible as in these Islands. Such problematic land could be brought under cultivation if appropriate system of reclamation/amendments and or genetic management through development and deployment of tolerant varieties are evolved and practiced. However, this is not too simple and easy problem. Massive efforts were mounted in this endeavour, however, the success rate is found to be moderate. During the last 40 years 30 salt tolerant rice varieties were released in Indian context and most of them are found to be moderately yielding. It is very difficult to compromise between high salt tolerance and high yielding ability. Since salt tolerance is a polygenic character, it is a challenging task to pyramid all the gene(s) or alleles in a single stock. Status of phenotypic marker were also found to be highly empirical and a large number of edaphic factors interplay with salinity stress.

Thus due to lack of suitable selection criteria the development of highly salt tolerant high yielding varieties in rice remain as an incomplete episode. In this cross road the molecular markers finds appropriate place to be used upon. Molecular markers have more potential; having better resolution and do not get affected by the changing environment in major cases. In the present study efforts were made to develop a F_3 mapping involving Pokkali × IR 28 with concurrent phenotyping in response to excess salt under artificially stimulated condition. This will be useful in tagging of genes for salinity tolerance in breeding programes.

MATERIALS AND METHODS

DNA Isolation

DNA was extracted from tender leaves following Murray and Thompson (1990) for SSR analysis. Young leaf tissue from F_3 plants were quick freezed in liquid nitrogen (-196 ^{0}C) and ground to a fine powder in pre-chilled autoclaved mortar and pestle. The ground powder was transferred to a sterile 5- ml centrifuge tube, incubated in water bath and mixed thoroughly. Chloroform: isoamyl alcohol treatment and centrifugation was carried out depending upon the purity of DNA

preparations and the upper aqueous phase was extracted several times with fresh chloroform: isoamyl alcohol. The final aqueous phase was transferred to another centrifuge tube to which ice-cold isopropanol was added and mixed gently. At this stage, DNA-CTAB complex was found to be precipitated as a whitish matrix and this DNA complex was pooled out with a bent Pasteur pipette. The pellet was washed and gently agitated for a few minutes and collected by centrifugation (10 min at 5000 × g, 4 ^{0}C). An appropriate volume of TE buffer was used to dissolve the pellet at 4 ^{0}C with agitation, overnight. The Collected DNA was purified and used for PCR using primers specific to SSR markers.

SSR Analysis

The PCR was performed using primers (specific to the SSR markers), *Taq* DNA polymerase, dNTP and DNA template. The PCR amplification reactions was carried out in a Peltier Thermal Cycler (MJ Research, PTC-200) with the following programme: one pre-cycle at 96ºC for 2 minutes; followed by 30 cycles at 94ºC for 1 minute, 56ºC for 1 minute and 72ºC for 1 minute; and a final elongation at 72ºC for 7 minutes. The allelic polymorphism was checked in denaturing polyacrylamide gels containing TBE buffer and visualized by Ethidium bromide staining. Amplicon profiling and data analysis was carried out in Molecular Analyst - Bio1D ++ ver. 99.04 software.

RESULTS AND DISCUSSIONS

Development of Mapping Population

F_1 Generation

F_1 hybrids of IR28 × Pokkali were previously grown in the net house (Fig. 1). Half of the randomly selected F_1 hybrids were self-pollinated to develop F_2 generation. F_1 plants were harvested individually. About 1250 seeds were collected from F_1 to grow F_2 population. The agronomic performance of F_1 is given in Table 3. The data showed that the F_1 population is more inclined towards Pokkali.

Table 2: Performance of Parent and F_1 Under Simulated Glasshouse Condition

Character	F_1 hybrid	Parent	
		IR28	Pokkali
Plant height (cm)	125.6	87.2	136.5
No. of tillers/ plant	7.25	10.52	4.32
Panicle length (cm)	22.06	23.92	18.95
No. of filled grains/ panicle	60.16	96.44	47.05
Spikelet sterility (%)	44.71	28.61	33.17
Grain yield/ plant (g)	12.21	24.11	5.69

F_2 Generation

The F_2 seeds grown plant were used to plot-progeny trial in the Experimental Farm, Bloomsdale of CARI, Port Blair (Fig. 2). The F_2 plants were harvested after plant wise at maturity. The agronomic performance of F_2 plants were recorded for physical characterization and to assess the distribution behaviour of F_2 segregating population. In the segregating generation (F_2), the individuals varied greatly among themselves (data not presented). From F_2 population 300 plants were randomly selected and single seed descended (SSD) method has been adopted to grow the F_3 population.

F_3 Generation

F_3 plants were initially transplanted in plastic pots in glass house condition. The plants at active tillering stage were split into two. One half was used for phenotyping under salinity stress and the remaining was grown under normal soil. In this study SSR profile of only twenty four plants has been employed and efforts were made to link SSR profile to salinity tolerance character. Nonetheless the result was found to be interesting though a population comprising only 22 F_3 segregants and resistant check Pokkali and susceptible check IR28 was studied in this investigation. The result showed ample polymorphism in respect of SSR primers, which is essential for a molecular marker programme in mapping gene of interest. Considering the degree of polymorphism it can be concluded that SSR may be used in tagging gene(s) for salt tolerance in rice with no hesitation. The dendrogram analysis of population revealed that the group was divided into two distinct groups. The upper group comprised of 8 lines and resembled IR28. The lower group consisted of 14 lines and resembled Pokkali. Efforts were made to link SSR profile with salt tolerance character as gleaned from phenotypic assessment of the segregants in response to salinity stress. The result can not be conclusive since the information obtained only for a part population involving 22 segregants only. However, a few bands in respect of RM 5638 and RM 7341 present in chromosome 1 were found to have bright possibility to be linked with salt tolerance character, which could be, confirmed when the entire information from all 150 mapping population would be compiled and interpreted. However, the present information suffice amply that SSR could be used as a reliable, easy to handle and reproducible marker system in molecular profiling of F_3 segregants. It is also envisioned that in tagging salt tolerant gene(s) SSR would be playing an important role in rice as gleaned from this study involving a part population of F_3 segregants.

Fig 1: A) IR28, B) IR28 × Pokkali, C) Pokkali

Fig. 2 :F_2 Population in Experimental Field

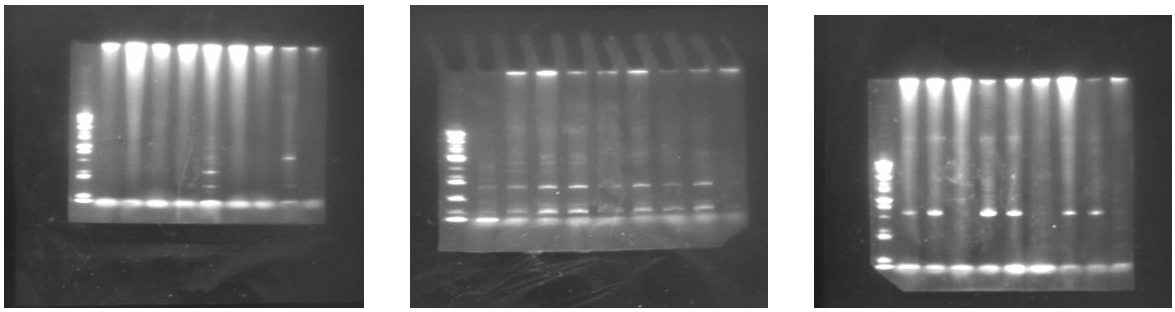

Fig 3: Microsatellite Analysis of a F_3 Population Involving RM 5638

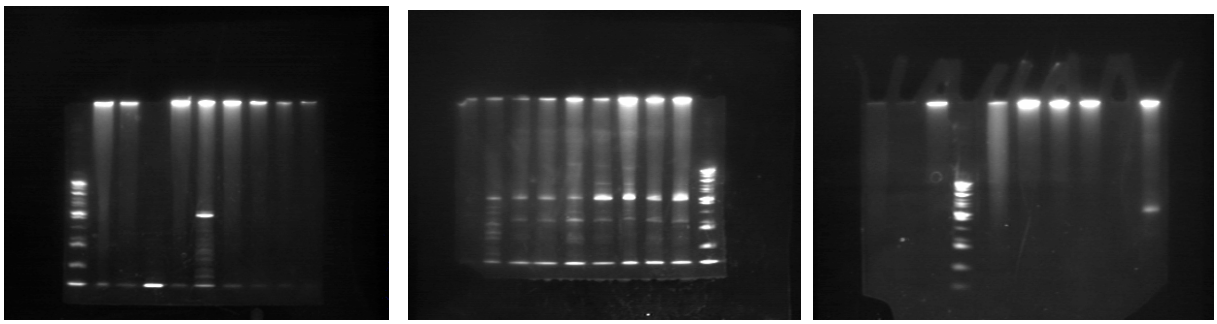

Fig 4: Microsatellite Analysis of a F_3 population Involving RM 7341

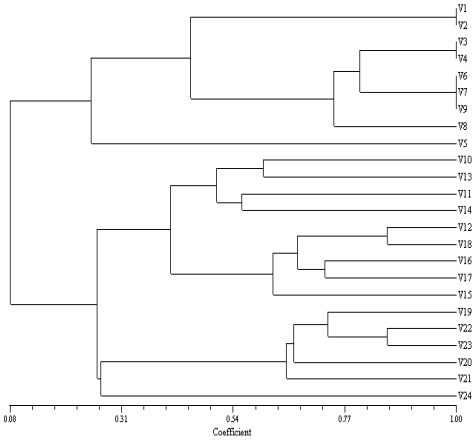

Fig. 5: Dendrogram Constructed with SSR Marker for the F_3 Population (V2 To V23) and Parent IR28 (V1) and Pokkali (V24)

REFERENCES

1. Abenes, M.L.P., Angeles, E.R., Khush, G.S., Andhuang, N. and Hunang, N. (1993) Rice Genetic Newsletter, 10, 120 –123.
2. Mohan, M., Nair, S., Bentur, J.S., Roa, U.P. and Bennett, J. (1994) Theor. Appl. Genet, 87, 782-788.
3. Paul, N.K. and Ghosh, P.D. (1986) Current Science, 55, 568-569.
4. Quarie S, Laurie D, Zhu J, Lebreton C, Scikhodskii A, Steed A, Witsenboer H, Calestani C. (1997) Plant Mol Biol. 35: 155-165.
5. Ronald, P.C., Albano, B., Tabien, R., Abenes, L. (1992) Mol. Gen Genet. 236, 113- 120.
6. Toenniessen, G.H. and Khush, G.S. (1991) Biotechnology in Agriculture, Vol. (6). Rice Biotechnology. CAB International/ IRRI, Wallingford, UK/Manila, Philippines, pp. 309-313.
7. Yadav, J.S.P. and Gupta, I.C. (1984) Usar Bhumi Ka Sundar (in Hindi), ICAR, New Delhi.
8. Murray, M.G. and Thompson, W.F. (1980) Nuc. Acid. Res, 8, 4321–4325.
9. Zapata, F.J., Alejar, M.S., Torrizo, L.B., Novero, A.Y., Singh, U.P. and Senadhira. (1991) Ther. Appl. Genet. 83: 6-11.
10. Mandal, A.B., Elanchezhian, R. and Mazumder, N.D. (2004). Genetic management for increased productivity of rice in Andaman and Nicobar Islands, Published by Central Agricultural Research Institute, Port Blair.
11. Raina, S.N., Rani. V., Kojima. T., Ogihara, Y., Singh, K.P. and Devarumath, R.M. (2001) Genome. 44:763-72. (Reddy et al., 2002)
12. Rivard, R., Cappadocia, M., Vincent, G., Brisson, N. and Landry, B.S. (1989) Theor. Appl. Genet. 78, 49-56.
13. Saikia, D., Khanuja, S.P.S., Shasany, A.K., Darokar, M.P., Kukreja, A.K. and Kumar, S. (2000) Plant Genetic Resources Newsletter, 121, 27-31.
14. Hulbert, S.H., Richter, T.E., Axtel, J.D. and Bennetzen, J.l. (1990) Proc. Natl. Acad. Sci. USA. 87, 4251-4255.

15. Sarma, N.P., Sundaram, R.M., Biradar, S., Ramesh, M.S., Viraktamath, B.C. and Sonti, R.V. (2002) Hybrid Rice Newsletter 3(1), 9.
16. Hosokawa, K., Minami, M., Kawahara, K., Nakamura, I. and Shibata.(2000) Planta Med. 66, 270-2.
17. Hulbert, S.H., Ilatt, T.W., Lagg, E.J., Lincoln, S.E., Lander, E.S. and Michelmore, R.W. (1988) Genetics: 947 – 958.
18. Jeffreys, A.J., Wilson, V., Thein, S. L.(1985) Nature. 314, 67-73.
19. Klein- Lankhore, R., Rietveld, P., Machiels, B., Verkerk, R., Weide, R., Gebhardt, C., Koornneef, M. and Zabel, P. (1991) Theor. Appl. Genet. 81, 661-667.